Seed Dormancy Imposed by Covering Tissues Interrelates to Shattering and Seed Morphological Characteristics in Weedy Rice

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ABSTRACT

Seed dormancy, a major adaptive trait in plants, facilitates the survival of weeds and provides for resistance to preharvest sprouting (PHS) in cereal crops. Seventeen weedy strains and 24 cultivars of rice (*Oryza sativa* L.) were evaluated for germinability to screen for donors of dormancy genes. Extremely dormant genotypes were identified from the weedy strains. These genotypes displayed hull- and pericarp/testa-imposed dormancy. Three dormant weedy strains, LD, TKN12-2, and SS18-2, were crossed and backcrossed with the nondormant breeding line EM93-1 to determine the relationship between dormancy and the shattering, awn, hull color, and pericarp/testa color characteristics. All these characteristics interrelated to the covering-imposed dormancy; the weedy forms of the characteristics significantly reduced germination in the BC_1F_1 populations. Moreover, multiple linear regression analyses revealed significant effects of interaction between the characteristics on dormancy in the populations. The interrelation and interaction reflect the importance of combined effects of dormancy and other weedy characteristics in the adaptation of weedy populations to agroecosystems, and suggest that domestication and breeding activities have eliminated dormancy alleles at loci near the genes for shattering and the morphological characteristics from improved cultivars.

WEEDS ARE ORGANISMS adapted to human disturbances (Harlan, 1965). Many characteristics contribute to the adaptation and persistence of weeds (Baker, 1974). For example, shattering enables weed seeds to escape harvest, and dormancy promotes survival in the soil seed bank. Other characteristics, such as grain type and awns in wild oat (*Avena fatua* L.) (Johnson, 1935; Simpson, 1992) and dark seed coat color in proso millet (*Panicum miliaceum* L.) contribute to weed persistence (Khan et al., 1996), but their adaptive significance has not been extensively examined.

Outcrossing frequently occurs between conspecific weeds and crops, sometimes yielding fertile hybrids (Harlan et al., 1973; Langevin et al., 1990). Outcrossing facilitates gene flow from crop to weed populations and initiates hybridization-differentiation cycles (Ladizinsky, 1985; Oka, 1988). It is these cycles that allow populations of weeds to generate types that are better adapted to compete with or mimic improved cultivars. For example, researchers are now concerned with gene flow between herbicide-resistant cultivars and conspecific weeds

such as rice to weedy or red rice (*Oryza* spp.) (Oard et al., 2000; Gealy et al., 2003; Zhang et al., 2003). Systematic research is required to understand how seed dormancy and other characteristics such as awns, hull and pericarp/testa colorations, herbicide resistance, and shattering influence fitness in weed/cultivar hybrid-derived offspring.

Weedy strains are hypothesized to be intermediate between wild species and cultivars, and thus weeds are considered as part of the primary gene pool for crop breeding (Harlan et al., 1973). It is assumed that weedy races harbor genes for tolerance to various adverse conditions, but their breeding potential has not been well explored (Harlan, 1965; Oka, 1988). New genetic and genomic approaches will facilitate discovery of novel genes in nondomesticated germplasm for major advances in crop improvement (Tanksley and McCouch, 1997). We are utilizing weedy rice as a genetic system to explore fundamental and applied aspects of seed dormancy because weedy strains generally have a higher level of dormancy compared with cultivars (Oka, 1988; Tang and Morishima, 1997). Additionally, rice is a model system with a small genome size (430 Mbp) that has been sequenced (Goff et al., 2002; Yu et al., 2002).

Weedy rice occurs in areas with and without wild rice relatives (*Oryza* spp.) (Oka, 1988). Previous research has characterized the level and type of seed dormancy for several weedy and cultivated genotypes (Gu et al., 2003). Seed dormancy was associated with the presence of an awn, and black hull or red pericarp/testa colorations in two weedy strain-derived F_2 populations, but the degree of association varied with the cross or genetic background (Gu et al., 2003). In this research, we screened additional weedy strains, and traditional and improved cultivars for dormant genotypes. Three strongly dormant weedy strains were crossed and backcrossed with a breeding line to synchronize the genetic background of dormancy genes. Thus, the objectives of this research were to use primary segregation populations (BC_1F_1s) to establish the co-adaptive relationships between dormancy and other weedy characteristics, and demonstrate how domestication and breeding have resulted in a reduction of seed dormancy.

MATERIALS AND METHODS

Plant Materials and Cultivation

Seventeen weedy strains and 24 cultivars of Asian rice (*O. sativa* L.) and four cultivars of African rice (*O. glaberrima* Steud.) were screened for strongly dormant and nondormant genotypes. The weedy strains originated from Bhutan (BT1Ac,

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Abbreviations: DAR, days of afterripening; PHS, preharvest sprouting; QTL, quantitative trait loci.

Table 1. Species, types, and phenotypic variation in weedy characters of the genotypes used in this research.

| Species | Types | Number of genotypes in each category (present:absent) | | | | Percentage germination‡ mean ± SD (range) |
		Shattering	Awn†	Black hull color	Red pericarp color	
O. sativa	weeds	17:0	10ˡ:7	11:6	16:1	11 ± 25 (0–91)
	Indica cultivars	0:16	6ˢ:10	0:16	1:15	58 ± 32 (4–98)
	Japonica cultivars	0:8	0:8	0:8	0:8	65 ± 21 (25–90)
O. glaberrima	cultivars	0:4	1ˢ:3	0:4	2:2	39 ± 39 (2–92)

† Superscripts ˡ and ˢ indicate that the genotypes have seeds with a long (>3 cm) and short (<1 cm) awn, respectively.
‡ Germination of nonafterripened intact seeds was evaluated at 30°C for 30 d.

BT2Cb, and BT8Ab), Brazil (W1713), China (C9587, C9588, C9589, and LD), India (W1670-14), Korea (C9541, S434, and Hapcheon 3), Nepal (C9520, TKN5-3, and TKN12-2), Thailand (SS18-2), and the USA (US1). The majority of weedy strains were selected based on their origin and morphophysiological characteristics (Suh et al., 1997; Tang and Morishima, 1997). The cultivars of Asian rice included 16 *O. sativa* subsp. *indica* [e.g., CO39 (PI 597037), Dular (PI 180061), EM93-1, IR36 (PI 408586), IR64 (PI 497682), Nanjing 11, N22 (PI 461046), Peta, Pokkali, PTB10 (PI 400144), Seri Raja (PI 233660), Taichung Native 1 (PI 400158) and Teqing (PI 536047)] and eight *O. sativa* subsp. *japonica* [i.e., Lemont (PI 475833), Mahsuri (PI 400130), Milyang23 (PI 464609), Moroberekan (PI 434632), Nipponbare (PI 514663), Taichung65 (PI 275428), Todorokawase (PI 458775), and WJX] cultivars or breeding lines. These cultivars or lines range from dormant to nondormant based on scattered reports in the literature (Chang and Tagumpay, 1973; Seshu and Sorrells, 1986; Siddique et al., 1988; Wan et al., 1997; Lin et al., 1998) or preliminary observations. The four African cultivars (*O. glaberrima*) are Kebra 60 (PI 69463), Kebra 80 (PI 369480), Saka (PI 369481), and NSGC5945 (PI 590414). All weedy strains display seed shattering and most of them have a long awn, a black hull color, and a red pericarp/testa color (Table 1). Several traditional cultivars, such as Pokkali, Kebra 60, and Kebra 80, retain the red pericarp and/or a short awn. The other cultivars or breeding lines have the domesticated form of the traits (Table 1).

The weedy strains and some cultivars were self-pollinated with a single plant selected for one or more generations to purify the genotypes before experimentation. Three strongly dormant weedy strains, LD, TKN12-2, and SS18-2, were crossed with the nondormant breeding line EM93-1 to develop backcross F_1 (BC_1F_1) populations. EM93-1 was chosen as the recipient parent to simplify the genetic background because it is insensitive to photoperiod and has extremely short growth duration under long daylengths (Gu et al., 2004a), which facilitated hybridization and synchronization of flowering time in the BC_1F_1 populations.

Plants were cultivated using a mixture of clay soil and Sunshine medium (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) in pots (28 cm diam. × 25 cm height) with one plant per pot. Plants were maintained in a greenhouse with day/night temperatures set at 29/21°C. Supplementary light was used as needed to maintain a 14-h photoperiod, except at the late tillering stage when a 10-h photoperiod was used to induce and synchronize flowering. Panicles from weedy strains and BC_1F_1 populations were covered with paper bags at 10 to 14 d after flowering; the bagged panicles were fixed with bamboo poles to prevent shattering due to shaking. Seeds were harvested at 40 d after flowering, with flowering date for a plant marked by emergence of the top of the first panicle from the leaf sheath. Freshly harvested seeds were dried for 3 d in the greenhouse and cleaned by removal of green or immature seeds. Dried seeds that had water content of about 12% were sealed in plastic containers and stored at −20°C to prevent afterripening.

Phenotypic Identification

Germination Assay

The degree of dormancy was measured by percentage germination. Initial germination experiments were focused on weedy strains and cultivars. Dried intact seeds without an afterripening treatment were placed in 24-well tissue culture plates with one seed per well. Each well was lined with a Whatman No. 1 filter paper and wetted with 0.25 mL of sterile deionized water. Seeds were incubated for 40 d in the dark at 30°C and 100% relative humidity. A contaminated seed was replaced with an uncontaminated one or discarded when it occurred at the early or late stage of incubation, respectively. Germination was judged by >3 mm expansion of the radicle or the coleoptile and was rated daily for 1 wk and every other day for the remaining period. There were about 24 seeds per treatment with three replications. For weedy strains and traditional cultivars, this experiment was repeated at least once using seeds harvested from the same or different seasons.

Germination for the weedy strains, cultivars, and BC_1F_1 populations also was evaluated using the intact seeds afterripened at room temperature (about 25°C) for 11 d or 1, 11, and 21 d. Only 11 d afterripening (DAR) was used for seeds from the weedy strains and cultivars because we found this period of time was appropriate to distinguish difference in degree of dormancy between the two categories of genotypes (Foley et al., 2003). About 50 seeds of three replications each were germinated in a 9-cm Petri dish lined with a Whatman No. 1 filter paper and wetted with 10 mL of sterile deionized water in the conditions as described above.

Weedy strains and cultivars with moderately to strongly dormant intact seeds were further evaluated for germination of nonafterripened caryopses (dehulled seeds), caryopses with the pericarp/testa removed, and excised embryos to estimate the effect of tissue components on dormancy. Caryopses, caryopses with the pericarp/testa removed, and excised embryos were obtained and evaluated for germination as previously described (Gu et al., 2003). Germination period was 4 wk for caryopses and 1 wk for pericarp/testa-removed caryopses and excised embryos. There were about 50 caryopses or 20 embryos per treatment with three replications. Caryopsis germination for the LD-derived BC_1F_1 population also was evaluated using the same method as that for the intact seeds, except that the sample size was 30 caryopses.

Seed Shattering and Morphological Characters

Seed shattering was quantified with the shattering rate on the basis of air-dried weight. Panicles were cut from the plant and immediately shaken gently for about 20 s over a container to collect shattered seeds, and then hand-threshed to collect the nonshattered seeds. The seeds were cleaned by removal of empty spikelets and dried in a greenhouse for 3 d. Shattering rate was calculated as percentage of the shattered to the total seed weight. The awn characteristic was quantified as the percentage of seeds with an awn in a random sample of 50

seeds from a plant and expressed as the mean of three samples. Black hull and red pericarp/testa colors were classified as present and absent, with the presence of black or red colors rated as 1 and the absence as 0.

Data Analysis

Primary germination data (y) collected from the BC_1F_1 populations at different DAR was transformed by $\sin^{-1}y^{1/2}$ for statistical analysis. The relationship between two characteristics was determined by a simple linear correlation (CORR) analysis (SAS Institute, 1999). Effects of the characteristics awn, black hull color, red pericarp/testa color, and seed shattering and the two-way interactions on seed dormancy were estimated based on the multiple linear regression model:

$$y_i = m + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{1.2}x_{1.2} +$$
$$b_{1.3}x_{1.3} + b_{1.4}x_{1.4} + b_{2.3}x_{2.3} + b_{2.4}x_{2.4} + b_{3.4}x_{3.4} + e_i$$
$$(i = 1 \text{ to } N),$$

where y_i is the mean germination of seeds at 1, 11, or 21 DAR from the ith plant; m is the mean of the model; x_1, x_2, x_3, and x_4 are the variables for percentage of seeds with an awn, hull color, pericarp color, and shattering rate, respectively; $x_{1.2}$, $x_{1.3}$, $x_{1.4}$, $x_{2.3}$, $x_{2.4}$, and $x_{3.4}$ are the interactions between variables x_1 and x_2, x_1 and x_3, x_1 and x_4, x_2 and x_3, x_2 and x_4, and x_3 and x_4, respectively; b_1, b_2, b_3, b_4, $b_{1.2}$, $b_{1.3}$, $b_{1.4}$, $b_{2.3}$, $b_{2.4}$, and $b_{3.4}$ are the partial regression coefficients corresponding to the regressor variables x_1, x_2, x_3, x_4, $x_{1.2}$, $x_{1.3}$, $x_{1.4}$, $x_{2.3}$, $x_{2.4}$, and $x_{3.4}$, respectively; e_i is the residual effect including the random error and the genetic effect that is not explained by the above main and two-way interaction effects; and N is the number of plants in the BC_1F_1 population. The regression (REG) analysis was complemented by the SAS procedure REG (SAS Institute, 1999). The variables retained in the final model were determined by stepwise selection at a probability level of 0.05.

RESULTS
Strength and Types of Seed Dormancy

It took 3 and 4 wk of incubation for nonafterripened dried seeds and caryopses, respectively, to approach their maximum germination potential (data not shown). However, the variances in cumulative germination of seeds and caryopses at 7 d accounted for about 95% ($r = 0.973$) and 73% ($r = 0.852$), respectively, of the variances at 30 d (Fig. 1). Apparently, the commonly
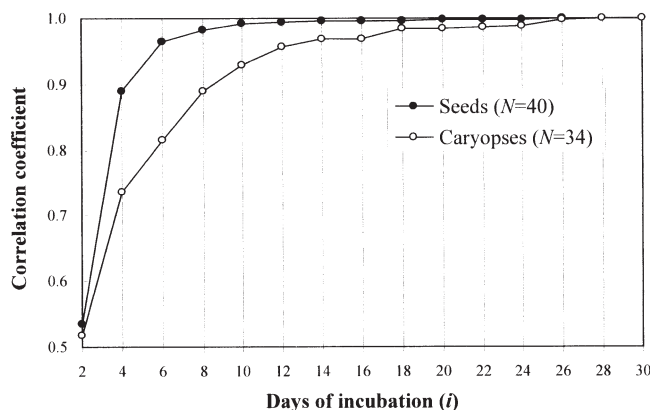

Fig. 1. Correlation between cumulative percentage germination at the ith ($i \le 30$) and the 30th day of incubation. N represents number of genotypes on which the correlation analysis was based.

used incubation time of 7 d is appropriate for evaluating dormancy with intact seeds from weedy strain-derived populations. Compared with intact seeds, caryopses were much more vulnerable to contamination, especially after several days of incubation. Thus, as a compromise and for consistency, we limited the germination period in our experiments to 7 d for both seeds and caryopses from the BC_1F_1 populations.

Mean germination of nonafterripened seeds was considerably lower in weedy strains than in African and Asian cultivars, although both the weedy and cultivated types contain dormant (<5% germination) and nondormant (90% or higher germination) genotypes (Table 1 and Fig. 2A). Compared with *japonica* cultivars, *indica* cultivars had a lower mean and a larger variation for germination percentage (Table 1). Seed germination for all cultivars and a few weedy strains increased following 11 DAR (Fig. 2A and B). With highly dormant seeds, an afterripening treatment is required to distinguish genotypic differences in dormancy strength and to realistically estimate genetic variance (Gu et al., 2003). The extremely dormant genotypes, defined by <5% germination at 11 DAR, were all weedy strains (Fig. 2B). These strains included LD, SS18-2, and TKN12-2 (Table 2). Of the remaining weedy strains, six exhibited strong dormancy (<35% germination at 11 DAR), the other two (BT1Ac and BT2Cb) showed weak and essentially no dormancy (73 and 92% germination at 11 DAR), respectively. Several traditional *indica* cultivars had strongly to moderately dormant seeds with 20 to 40% germination at 11 DAR. The degree of dormancy in some of the dormant cultivars, including N22, Peta, and PTB10, was similar to that in previous reports (Chang and Tagumpay, 1973; Seshu and Sorrells, 1986; Siddique et al., 1988). However, some previously reported dormant cultivars, such as Milyang 23 and IR36, displayed a relatively low level of dormancy in this experiment (data not shown). The remaining *indica* and all *japonica* cultivars had weak dormancy or nondormancy. The four African cultivars ranged from strongly or moderately to weakly dormant, to nondormant (Fig. 2B).

Dehulling nonafterripened seeds did not completely eliminate dormancy in all the dormant weedy strains and cultivars. Moderate to strong dormancy still occurred in 10 weedy strains including LD, and one African (Kebra 80) and three Asian (N22, Peta, and Pokali) cultivars as indicated by 8 to 55% germination of caryopses (Fig. 2C). Caryopses with the pericarp/testa removed and excised embryos from all the dormant genotypes displayed >90% germination after seven and four d of incubation, respectively (data not shown). These results support our previous observation that seed dormancy is imposed by the covering tissues of the hull and/or pericarp/testa in the weedy rice strains that have been evaluated to date (Gu et al., 2003).

Interrelation between Dormancy and the Other Weedy Characteristics

A wide range of variation in degree of seed or caryopsis dormancy was observed in the weedy strain-derived
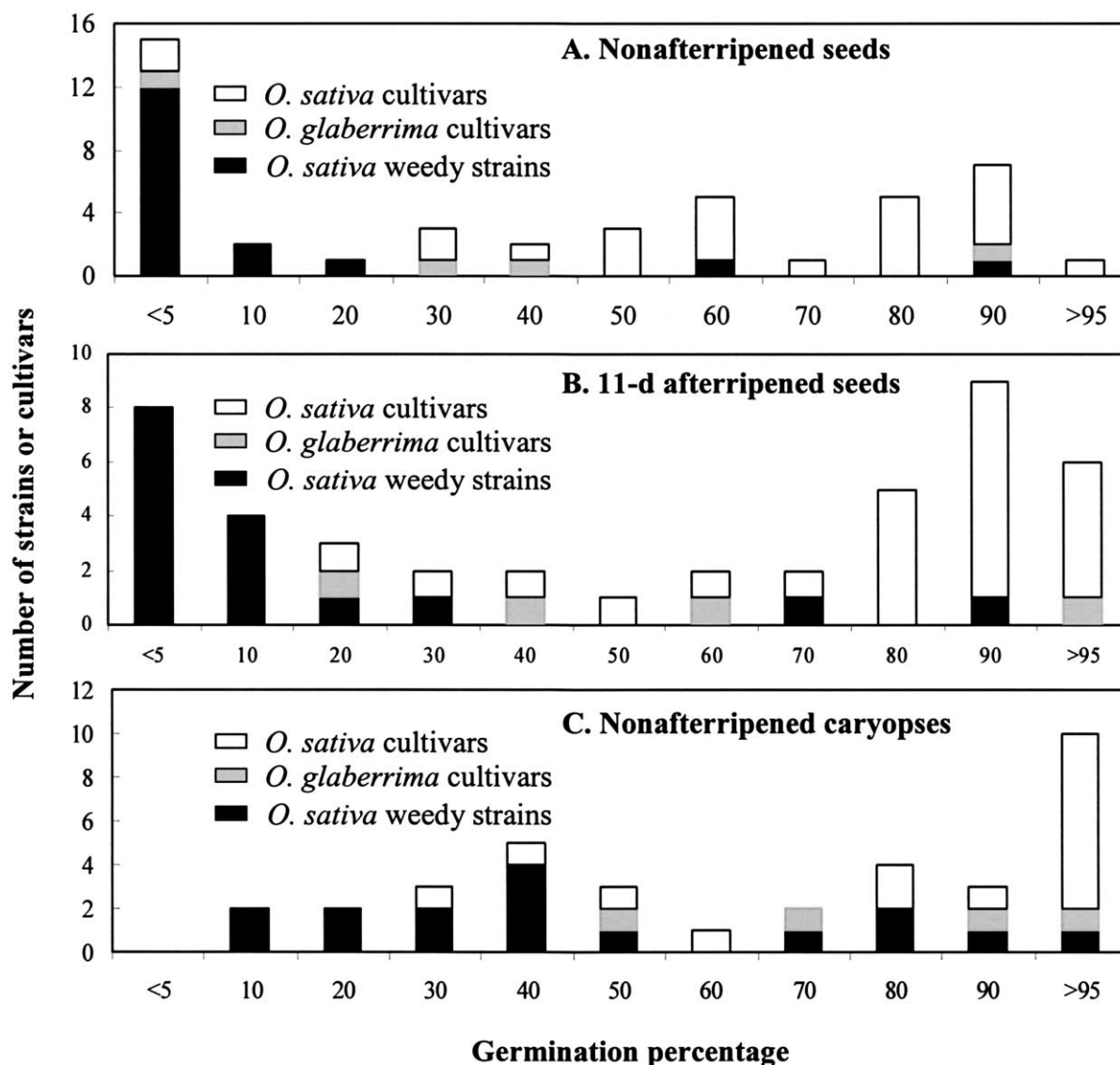
Fig. 2. Distribution for germination of (A) nonafterripened seeds, (B) 11-d afterripened seeds, and (C) nonafterripened caryopses for genotypes from weedy and cultivated rice. Only the moderately to strongly dormant genotypes were further evaluated for caryopsis germination.

BC$_1$F$_1$ populations (Table 2). The seed shattering and awn characteristics, as expressed by the shattering rate and percentage of seeds with an awn, also displayed continuous variation (Table 2). The black hull and red pericarp/testa color characteristics were relatively easy to group into presence and absence categories (Table 2).

Table 2. Germination of seeds at different days of afterripening (DAR) or nonafterripened caryopses and the characteristics shattering, awn, and black hull and red pericarp colors in weedy strain-derived BC$_1$F$_1$ populations.

Populations/parents†	Seed germination mean (range)			Caryopsis germination	Shattering rate	Awned seeds	Hull color ratio	Pericarp color ratio
	1 DAR	11 DAR	21 DAR					
	%							
BC$_1$F$_1$s								
EM93-1//EM93-1/LD	21 (0–67)	43 (1–89)	61 (1–99)	75 (5–100)	61 (3–92)	31 (0–100)	86:78§	51:113¶
EM93-1//EM93-1/SS18-2	20 (0–85)	55 (6–98)	74 (10–100)	NA‡	55 (3–95)	33 (0–100)	135:63	91:107
EM93-1//EM93-1/TKN12-2	12 (0–68)	57 (14–98)	67 (17–100)	NA	65 (8–100)	0 (0–0)	45:37	27:55
Parents								
EM93-1	76	96	98	97	4	0	straw	white
LD	0	1	6	11	95	100	black	red
SS18-2	0	1	3	83	91	100	black	red
TKN12-2	0	0	1	77	100	0	black	red

† EM93-1 is a breeding line; LD, SS18-2, and TKN12-2 are weedy strains originating from China, Thailand, and Nepal, respectively.
‡ NA, germination was not evaluated because of the relatively small difference between the parents.
§ Observed ratio of the plants with and without black hull colors in the population.
¶ Observed ratio of the plants with and without red pericarp color in the population.

Table 3. Correlation between percentage germination of seed or caryopsis and the characteristics of awn, black hull and red pericarp colors, and shattering in the weedy strain-derived BC₁F₁ populations.

Populations	DAR or caryopsis†	Pearson correlation coefficients			
		Hull color	Pericarp color	Shattering rate	Percentage seeds with awn
EM93-1//EM93-1/LD	1	−0.338***	−0.499***	−0.283***	−0.378***
	11	−0.444***	−0.493***	−0.187*	−0.397***
	21	−0.483***	−0.460***	−0.301***	−0.417***
	Caryopses	−0.280***	−0.498***	−0.017ns	−0.152ns
EM93-1//EM93-1/SS18-2	1	−0.208**	−0.131ns	−0.204**	−0.390***
	11	−0.254**	−0.196**	−0.194**	−0.435***
	21	−0.231**	−0.167*	−0.157*	−0.420***
EM93-1//EM93-1/TKN12-2	1	−0.417***	−0.367***	−0.570***	NA‡
	11	−0.587***	−0.457***	−0.508***	NA
	21	−0.623***	−0.497***	−0.443***	NA

* Significant at the $P = 0.05$ level.
** Significant at the $P = 0.01$ level.
*** Significant at the $P = 0.001$ or < 0.0001 levels.
ns, nonsignificant at the $P = 0.05$ level.
† DAR, days of afterripening before the seed germination; caryopsis, germination of nonafterripened caryopses.
‡ NA, not applicable because of no awn variation in the population.

All four characteristics were significantly correlated with seed dormancy in the three populations, with the presence of black hull color, red pericarp color, a high percentage of seeds with an awn, and a high shattering rate reducing germination (Table 3). Caryopsis dormancy in the LD-derived BC₁F₁ population was significantly correlated with red pericarp and black hull color, but not with shattering rate or percentage seed with an awn (Table 3), which is similar to an observation made for the LD/WYJ-derived F₂ population (Gu et al., 2003). The variance in degree of dormancy explained by the linear relationship varied with the characteristic. For example, red pericarp color in LD-, awns in SS18-2-, and black hull color and shattering in TKN12-2-derived populations accounted for about 25% ($r \approx -0.5$), 16% ($r \approx -0.4$), and 25% ($r = -0.4$ to -0.6) of the phenotypic variation in seed or caryopsis germination, respectively.

Multiple linear regression analysis detected significant main and two-way interaction effects on seed dormancy in all BC₁F₁ populations and a main effect of red pericarp color on caryopsis dormancy in the LD-derived population (Table 4). However, the occurrence of the main and interaction effects on seed dormancy varied with the characteristic and population. For example, a significant main effect of shattering was not detected in the three populations, and the main effects of black hull and red pericarp colors were not significant in the SS18-2-derived population. Black hull color influenced seed dormancy by interacting with the awn, red pericarp color, or shattering characteristics in the populations (Table 4). Theoretically, the variation between the populations and characteristics is because one or more of the four variables in the linear model do not independently influence seed dormancy.

Correlation analysis detected significant positive associations between any two of the three characteristics (awn, black hull color, and shattering) in the three weedy strain-derived BC₁F₁ populations (Fig. 3). Red pericarp color appeared weakly correlated to black hull color and independent of the awn and shattering characteristics in these populations (Fig. 3). The results from the correlation analysis confirm that the main and/or interaction effects of a characteristic on seed dormancy could be masked by or included in its interrelated characteristic(s).

DISCUSSION

The most dormant genotypes were weedy strains rather than the Asian and African cultivars. There appear

Table 4. Summary of the components retained in the multiple linear regression model based on the data from the BC₁F₁ populations.

Populations	DAR or caryopsis†	Intercept (*m*) and partial regression coefficients‡							
		m	*b₁*	*b₂*	*b₃*	*b₁.₂*	*b₁.₄*	*b₂.₃*	*b₂.₄*
EM93-1//EM93-1/LD	1	0.554***	−0.247***	ns	−0.227***	ns	ns	ns	ns
	11	0.833***	ns§	ns	−0.134*	−0.309***	ns	−0.158*	ns
	21	1.087***	ns	−0.132**	ns	ns	−0.291***	−0.162*	ns
	caryopses	1.247***	ns	ns	−0.252***	ns	ns	ns	ns
EM93-1//EM93-1/SS18-2	1	0.496***	−0.293***	ns	ns	ns	ns	ns	ns
	11	1.006***	−0.344***	ns	ns	ns	ns	−0.132**	ns
	21	1.227***	−0.315***	ns	ns	ns	ns	−0.102*	ns
EM93-1//EM93-1/TKN12-2	1	0.424***	NA¶	ns	−0.213***	ns	ns	ns	−0.118**
	11	1.138***	NA	−0.270***	−0.220***	ns	ns	ns	ns
	21	1.226***	NA	−0.300***	−0.170**	ns	ns	ns	ns

* Significant at the $P = 0.05$ level.
** Significant at the $P = 0.01$ level.
*** Significant at the $P = 0.001$ or < 0.0001 levels.
† DAR, days of afterripening before the seed germination; caryopsis, germination of nonafterripened caryopses.
‡ See the description in Materials and Methods for the linear model and the meanings of the partial regression coefficients.
§ ns, nonsignificant at the $P = 0.05$ level.
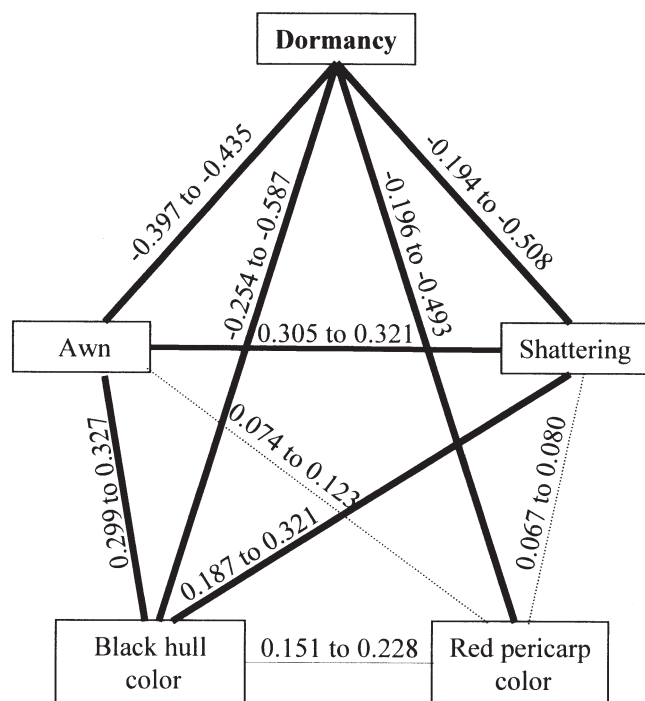¶ NA, not applicable because of no awn variation in the population.

Fig. 3. Interrelationship among seed dormancy, seed shattering, awn, black hull color, and red pericarp color characteristics. Dormancy was evaluated by germination of intact seeds at 11 d of afterripening. The number on each line represents the linear correlation coefficient range based on the three BC_1F_1 populations (Table 2). The fine line, heavy lines, and dotted lines indicate correlations are significant at the $P = 0.05$, $P < 0.01$, and nonsignificant at $P = 0.05$ levels, respectively.

to be some novel alleles underlying the strong dormancy in weedy rice that are not present in domesticated cultivars. Nondormant and weakly dormant genotypes also were identified in weedy strains (Fig. 2A), such as BT1Ac and BT2Cb, respectively. These weakly dormant or nondormant strains are similar to cultivars in a number of morphological characteristics. For example, both BT1Ac and BT2Cb have a straw-colored hull and awnless seeds, and BT2Cb also has a white-colored pericarp/testa. Considering their morphological characteristics and degree of dormancy, it is possible that the original seeds of these strains were collected from nondormant or weakly dormant variants in segregation populations derived from hybridization between cultivars and their accompanying weedy plants.

Only seed-covering (i.e., hull- and/or pericarp/testa)-imposed dormancy was identified in the weedy strains. This is consistent with previous research (Gu et al., 2003). The covering tissues have been hypothesized as a physical barrier to germination of dormant seeds, or they may contain inhibitors of germination (Bewley and Black, 1982). Removal of the hull and/or pericarp/testa alters the environment of the embryo (Leather et al., 1992). As such, germination inhibitors might be partly released from the endosperm or embryo, or embryo excision could induce production of chemicals in the embryo that stimulate germination. Therefore, we cannot rule out the existence of embryo or endosperm-imposed dormancy in weedy rice, which has been sug-

gested for wild rice (*Oryza* spp.) (Takahashi, 1963). We are developing near-isogenic lines using some of the extremely dormant genotypes to determine mechanisms underlying seed dormancy.

Dormancy of intact seeds interrelates to shattering, awn, black hull, and red pericarp/testa color characteristics in weedy rice (Table 3). However, not all of these weedy characteristics were significantly correlated with seed dormancy in F_2 populations derived from crosses between different weedy strains and cultivars (Gu et al., 2003). Thus, we used a backcross strategy with the breeding line EM93-1 as the recipient genetic background and several different weedy strains to reexamine the relationship of weedy characteristics to seed dormancy. The three weedy strain-derived BC_1F_1 populations yielded similar correlations between the characteristics and seed dormancy (Table 3). In fact, seed dormancy has been associated with grain type in populations from wild oat/cultivated oat crosses (Johnson, 1935), with red grain color in wheat (*Triticum aestivum* L.) (Gfeller and Svejda, 1960), and with seed shattering in rice wild relative (*O. rufipogon* Griff.) (Oka, 1988; Cai and Morishima, 2000). In all cases, presence of the weedy or wild-type characters enhances the degree of dormancy. Our research supports a conclusion that seed dormancy is a major but not independent factor contributing to adaptation of weeds. Seed dormancy promotes the survival of genotypes with other weedy characteristics, and in turn, the other characteristics help accumulate and maintain dormancy genes in nondomesticated populations. This conclusion has been implied by the concept of *adaptive* or *domestication syndrome* developed in crop evolution research (Harlan et al., 1973; Oka, 1988). It is well known that seed shattering and dormancy are extremely important adaptive traits in seed-bearing plants in nature. However, morphological characters such as awns, and hull and pericarp/testa colors also have adaptive significance because of their association with both shattering and dormancy. Figure 3 summarizes the relationship between the characters examined in this research.

Previous research focused mainly on the influence of one weedy characteristic on seed dormancy. However, combined effects of shattering and morphological characteristics on seed dormancy should not be neglected in nondomesticated populations. The significant two-way interactions detected in the BC_1F_1 populations (Table 4) are a simple example. The interaction effects indicate that dormancy genes are better preserved in variants with more than one weedy characteristic in weedy populations. The positive impacts of multiple factors on one another (Fig. 3) are probably near to the natural condition required for survival or persistence of weeds in agroecosystems. Previously, we identified six quantitative trait loci (QTLs) and different epistases that regulate seed dormancy in the SS18-2-derived population (Gu et al., 2004b). The QTL qSD^s7-1 is linked to a red pericarp/testa color gene in this population. It is likely that additional dormancy loci link to genes for other adaptive traits, as reported in an accession of *O. rufipogon* (Cai and Morishima, 2002). As a practical

matter when considering the impacts of multiple factors, transfer of herbicide resistance transgenes occurs under natural conditions from rice cultivars to weedy rice (Oard et al., 2000; Zhang et al., 2003), fitness of the weed might be increased, particularly if the transgene is closely linked with one or more genes for other adaptive traits.

Domestication and breeding processes certainly lead to elimination of dormancy alleles, especially at loci near to genes for other weedy characteristics. Some traditional cultivars of rice retain some wild-type characteristics such as a short awn and red pericarp/testa color (Table 1), but improved cultivars lack these wild-type or weedy traits. More QTLs for seed dormancy or PHS have been reported for wild and weedy rice (Cai and Morishima, 2000; Gu et al., 2004b) than for cultivars (Lin et al., 1998; Dong et al., 2003). Selection during early domestication might have focused more on elimination of seed shattering and other undesirable seed morphological characters than on dormancy, or selection for the domesticated form of these traits may have incidentally selected genotypes with a low level of seed dormancy due to such interrelations and interactions detected in this experiment (Fig. 3 and Table 4). Domestication reduced the genetic diversity of crops, and breeding activities have been accelerating this reduction (Tanksley and McCouch, 1997). The lost diversity can be partly regained from weedy strains to solve practical problems like PHS. Phenotypic associations such as those in this report can result from either genetic linkage or pleiotropic effect. Thus, fine mapping or cloning the loci responsible for interrelated characteristics is required to answer the question: Can dormancy genes whose phenotypes associate with the other weedy characteristics be employed to improve the resistance to PHS?

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